No evidence for a prethrombotic state in stable chronic inflammatory bowel disease

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SUMMARY Ulcerative colitis and Crohn’s disease are associated with a high risk of thromboembolic complications. The questions whether reported risk factors such as low antithrombin III concentrations, thrombocytosis and spontaneous platelet aggregation are merely related to the activity of the inflammatory process remains to be answered. Therefore we investigated 40 patients with an established colitis or Crohn’s disease, without signs of active inflammation (normal history, normal ESR and leucocyte count). Of these patients only one patient revealed thrombocytosis, six patients spontaneous platelet aggregation. All patients had normal β-thromboglobulin and platelet factor 4 plasma levels. No other prethrombotic abnormalities were encountered. There was normal factor VIII C (increased in three patients), normal VIII C/VIII R Ag ratio (1·2), antithrombin III, normal plasminogen and normal α2-antiplasmin. Normal fibrinopeptide A and Bβ (15-42) plasma levels (n = 15) in these patients excluded in vivo thrombin or plasmin generation. We conclude that stable chronic inflammatory bowel disease is in general not associated with prethrombotic coagulation abnormalities.

The course of inflammatory bowel disease is variably complicated by thromboembolic disease. The reported incidence varies between 1·2 and 39%. This wide range is due to lower incidences reported in clinical studies (1·2–7·5%)1–3 versus higher incidences in necropsy studies (6·6–39%).4 The obvious bias in these studies is the selection procedure of patients. Most reports concern severely ill patients in an active terminal stage of inflammatory bowel disease.

That inflammatory bowel disease predisposes to thromboembolic disease is weakly substantiated by studies claiming a wide range of coagulation and platelet abnormalities suggestive of a “prethrombotic state.” Only a few investigations report on factors implying a real risk for thromboembolism, such as an acquired antithrombin-III deficiency.5

Deficiency of this important coagulation inhibitor is generated in inflammatory bowel disease by intestinal protein loss. Due to modern diagnostic means and increased therapeutic potential severely ill inflammatory bowel disease patients are now less frequently admitted. Also a reduction of thromboembolic complications have been observed in these patients.

It is therefore of interest to reinvestigate inflammatory bowel disease patients in a defined stable condition using selected methods which provide data on in vivo activation of the coagulation system and/or of platelets.

Material and methods

Forty inflammatory bowel disease patients (mean age 33·9 yr, range 17–54 yr) under control of our outpatient department of gastroenterology were studied. Twenty-six patients were diagnosed as having ulcerative colitis and 14 Crohn’s disease. The diagnosis was made on clinical criteria and confirmed by x-ray, endoscopy and histology on biopsy specimen.

All patients were in a stable remission phase of their disease. This was determined by using the activity index of van Hees et al* and of Truelove et al.+ Patients with Crohn’s disease were considered to be in the non-active phase with an index value below 100, or considered mild in the case of ulcerative colitis.

Of the group of patients with ulcerative colitis, 17 patients had local involvement of the colon, six
patients had involvement of the entire colon and three patients had only rectum lesions. Two patients underwent surgery; proctocolectomy, hemicolec-
tomy respectively.

Of patients with Crohn's disease, seven had lesions on the distal part of ileum, two patients had lesions in the colon as well as in the ileum, three had Crohn's disease of the colon and two patients had anorectal lesions. Four patients of this group had small bowel resection in their history, and one patient underwent a hemicolecotomy.

Ten ulcerative colitis patients and five Crohn's disease patients did not receive any therapy. The remaining patients received salazopyrine and corticosteroids.

Besides the general patient history, a detailed case history on thromboembolic complications was obtained. Additional clinical information and informed consent were obtained from all patients prior to the study.

**LABORATORY INVESTIGATIONS**

*General coagulation and fibrinolytic tests*

Venous blood was collected using a 20 gauge Wasterman needle in plastic tubes containing either solid K$_2$EDTA (1·5 mg/ml blood) or trisodiumcitrate dihydrate 3.2% (one volume to 9 volumes of blood).

Plasma was prepared by centrifugation for 10 min at 1700 g followed by a second run at 12000 g. EDTA-plasma was used for the automated chromogenic determination of factors II, X, anti-
thrombin III, plasminogen and $\alpha_2$-antiplasmin.$^{10-14}$ Citrate-plasma was used for the assay of factors V, $^{15}$ VIIIC,$^{16}$ VIIIR $\text{Ag}^{17}$ and fibrinogen.$^{18}$

A plasma mixture (40 donors, sex ratio 1:1) was the reference for the one stage factor V and VIIIC coagulation assays. Normal ranges for all assays were obtained in 176 ostensibly healthy subjects (mean age 35 yr, range 10–62 yr).

**Tests measuring activated coagulation and fibrinolytic pathways: fibrinopeptide A and Bβ 15-42**

Blood (2·7 ml) was collected in polystyrene tubes containing 0·3 ml heparin (1000 U/ml, Kabi Vitrum, Stockholm, Sweden) and Trasylolr$^R$ (1000 U/ml, Bayer, Leverkusen, FRG) in 3·8% trisodium citrate dihydrate. Plasma was prepared immediately by centrifugation at 4°C followed by precipitation in duplicate of 0·4 ml in 0·4 ml iccold polyethylene-glycol (PEG 6000, Merck FRG) 40% in phosphate-buffered saline. Supernatants were heated for 2 min at 100°C to precipitate residual traces of fibrinogen. The radioimmunoassay on the supernatant for fibrinopeptide A was essentially per-

formed as described.$^{19}$

All samples were analysed using antiserum 44656 and antiserum 6216 with different specificity which will be published in detail elsewhere. Their reactiv-
ity with FPA (Aα 1-16) is identical and taken as 100%, then their reactivity with fibrinogen is ∼30% and < 0·5%, with plasmin-induced fibrinogen fragments 30–80% and < 0·5%, with granulocytic proteases-induced fibrinogen fragments 30–100% and 0–3% respectively.

Concentrations in 60 healthy control subjects were in a range of 0–2 ng/ml. The peptide Bβ 15-42 was measured after the PEG precipitation pro-
cedure using the commercial kit (batch B 132-1, IMCO Corporation, Stockholm, Sweden).

**Platelet tests**

Platelets were counted using the Coulter Cell counter 134 (Analytic Instruments, Sweden).

Spontaneous platelet aggregation and aggregation upon challenging with 0·1 µg/ml adenosine diphosph-(ADP) were performed as described before.$^{20}$

The platelet release products β-thromboglobulin ($\beta$-TG) and platelet-factor-4 were measured in plasma according to Ludlam and Cash$^{21}$ using commercially available RIA test kits (Radiochemical Centre, Amersham, Bucks).

**Results**

One patient with ulcerative colitis had a history of a pulmonary embolism during the period of bedrest after proctocolectomy. At that time he was treated with subcutaneous heparin as antithrombotic prophylaxis.

One patient developed deep vein thrombosis of his leg during an exacerbation of his Crohn's disease.

The other patients experienced no such complications. The inflammation related parameters ESR, haemoglobin, leucocyte counts and albumin concentrations were normal in all patients and confirmed the stable remission phase.

**General coagulation and fibrinolytic parameters**

All coagulation factors (Table) were within the normal range except in three patients who had mild deficiencies of factor II (0-62 U/ml), factor X (0·64 U/ml) and factor V (0·66 U/ml) respectively.

Three patients had raised concentrations of factor VIIIC. The mean ratio F VIIIC/factor VIIIR $\text{Ag}$ was 1·2 (ratio in-control subjects 1:1). Antithrombin III and fibrinolytic parameters were in the normal range except in one patient who had a slightly decreased plasminogen level.
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Coagulation, fibrinolytic parameters and platelet count and function in patients with inflammatory bowel disease

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean values</th>
<th>Units</th>
<th>Range</th>
<th>n</th>
<th>Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor II</td>
<td>1.03</td>
<td>U/ml</td>
<td>0.62-1.62</td>
<td>40</td>
<td>0.70-1.40</td>
</tr>
<tr>
<td>Factor X</td>
<td>1.03</td>
<td>U/ml</td>
<td>0.64-1.49</td>
<td>40</td>
<td>0.70-1.40</td>
</tr>
<tr>
<td>Factor V</td>
<td>0.96</td>
<td>U/ml</td>
<td>0.66-1.80</td>
<td>39</td>
<td>0.80-1.40</td>
</tr>
<tr>
<td>Factor VIIIC</td>
<td>1.11</td>
<td>U/ml</td>
<td>0.77-2.50</td>
<td>35</td>
<td>0.50-1.50</td>
</tr>
<tr>
<td>Factor VIIIIRAg</td>
<td>0.94</td>
<td>U/ml</td>
<td>0.37-2.16</td>
<td>38</td>
<td>0.40-1.80</td>
</tr>
<tr>
<td>AT-III</td>
<td>1.04</td>
<td>U/ml</td>
<td>0.77-1.28</td>
<td>40</td>
<td>0.80-1.40</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>1.06</td>
<td>U/ml</td>
<td>0.64-1.45</td>
<td>40</td>
<td>0.70-1.40</td>
</tr>
<tr>
<td>( \alpha_2 )-antiplasmin</td>
<td>1.01</td>
<td>U/ml</td>
<td>0.78-1.35</td>
<td>40</td>
<td>0.80-1.40</td>
</tr>
<tr>
<td>Fibrinopeptide A antiserum (44656)</td>
<td>0.19</td>
<td>pmol/ml</td>
<td>0.0-0.8</td>
<td>15</td>
<td>&lt; 1.3</td>
</tr>
<tr>
<td>Fibrinopeptide A antiserum (6216)</td>
<td>0.19</td>
<td>pmol/ml</td>
<td>0.0-0.8</td>
<td>15</td>
<td>&lt; 1.3</td>
</tr>
<tr>
<td>Fibrinopeptide B( \beta ) (15-42)</td>
<td>Undetectable</td>
<td>pmol/ml</td>
<td></td>
<td>15</td>
<td>&lt; 0.72</td>
</tr>
<tr>
<td>( \beta )-thromboglobulin</td>
<td>36-63</td>
<td>ng/ml</td>
<td>2.9-49.81</td>
<td>15</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Platelet factor 4</td>
<td>8.07</td>
<td>ng/ml</td>
<td>0.24-13.56</td>
<td>15</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>Platelet count</td>
<td>229-0</td>
<td>\times 10^9/l</td>
<td>120-400</td>
<td>40</td>
<td>150-350</td>
</tr>
</tbody>
</table>

Test measuring activated coagulation and fibrinolytic pathways

Fibrinopeptide A with both antisera and \( \beta \beta \) 15-42 showed normal levels in the 15 patients investigated (Table).

Platelet count and functions

Only one patient showed a mild thrombocytosis. Spontaneous platelet aggregation was encountered in six patients who showed also hyperaggregation upon challenging with ADP.

In 15 patients \( \beta \)-TG and platelet-factor-4 levels were within the normal range (Table).

Discussion

Thromboembolism is still an accepted complication of inflammatory bowel disease. Main causes are considered to be dehydration, immobilisation and activity of the disease. Whether a so-called hypercoagulative state contributes remains to be solved.

Thus far several investigators have demonstrated increased levels of factor V, VIII and of fibrinogen.\(^6\)\(^7\)\(^8\)\(^9\) It is most uncertain that this implies an increased risk for thrombosis.

Other studies revealed an acquired deficiency of antithrombin-III in active inflammatory bowel disease patients which was found to return to normal upon clinical remission.\(^9\) At present this is the only real risk factor for thrombosis thus far reported.

Acquired antithrombin-III deficiency has indeed an associated risk for spontaneous thrombotic events.\(^3\)\(^2\) In order to establish the occurrence of risk factors for thromboembolic disease we investigated inflammatory bowel disease patients in a stable remission of their disease as judged by activity indices.\(^8\)\(^9\) We did not observe major changes in coagulation, fibrinolytic factors and of the respective inhibitors antithrombin-III and of \( \alpha_2 \)-antiplasmin. Since conventional coagulation assays do not necessarily reflect activation of blood coagulation, sensitive tests for the detection of activation of blood coagulation such as fibrinopeptide A and of fibrinopeptide \( \beta \beta \) for the detection of plasmin activity in vivo were employed. All patients showed normal levels of fibrinopeptide A (Fpa) immunoreactivity, as determined with both antisera. This excluded substantial intravascular thrombin activity or influx of Fpa (\( \alpha \alpha -16 \)) from the inflammatory site into the circulation. In addition, as antiserum 6216 cross-reacts with plasmin or granulocytic protease induced fibrinogen fragments, a major contribution of these enzymes to intravascular and presumably extravascular fibrinogenolysis becomes unlikely.

The observation of the absence of \( \beta \beta \) (15-42) immunoreactivity in the plasma of these patients strengthens this conclusion.

Thrombocytosis and shortened platelet survival in inflammatory bowel disease may be considered as a risk factor for arterial thrombotic complications such as stroke.\(^5\)\(^7\)\(^2\)\(^3\)-\(^2\)\(^7\)

Spontaneous platelet aggregation was found in six of 40 patients, which is in agreement with other observations.\(^2\) This finding is possibly relevant for the pathogenesis of arterial thrombotic events, but of most uncertain relevance for the pathogenesis of venous thromboembolism. Also tests for blood platelet activation c.q. circulating platelet release products were included in this study and showed no raised plasma levels of \( \beta \)-TG or platelet-factor-4 which excludes excessive intravascular consumption of platelets.

In conclusion it should be stressed that inflammatory bowel disease should be considered in two separate phases, the acute active and the non-active phase. From our data in patients in the non-active phase of their disease it is concluded that no evidence for the existence of a prethrombotic state is demonstrated.
References


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